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قسم البحوث البيئية والصحية

تقرير بقايا مضادات الجراثيم في ذبائح الهدي والأضاحي

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Antibacterial drug residues in carcasses of animals slaughtered in Haj

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Summary

A survey study was carried out for detection of antibacterial residues in muscles and liver samples obtained from sacrificed slaughtered camels, cattle and sheep during the season of Haj (1423H). Out of forty sacrificed slaughtered camels, one muscle sample (2.5%) was contained unidentified inhibitory substance, while two liver samples (5%) were contained each of Sulphaquinoxaline Sodium (SQNa) and Chlorampheincol (CAP) with concentrations of 0.04 and 0.03 mg/kg, respectively. Concerning to sacrificed slaughtered sheep, muscles were free from antibacterial residues, while one liver sample (2.5%) was contained each of SQNa (with concentration of 0.02 mg/kg) and unknown inhibitory substance. No antibacterial residues could be detected in any of the samples taken from sacrificed carcasses of cattle. However, the SQNa and CAP residues detected in this study were all below the Maximum Residual Limits (MRLs) reported in the Codex Alimentarius of World Health Organization (WHO).

Introduction

Several products are currently licensed for use in different veterinary fields in the Kingdom of Saudi Arabia. Of these products, antibacterial drugs, insecticides and hormones are the most common ones and there fate within the animal body is highly variable. For example, some compounds such as prostaglandin's are eliminated from the body very quickly while others such as antibiotics, are much more persistent.

It is well known that antibacterial residues in human foods of animal origin represent great hazards for public health due to many deleterious effects. Of these effects aplastic anemia, acute leukemia, bone marrow injuries. Another drawback is the possible generation of bacterial strains of multiple resistance to the most common

drugs with failure of treatment of many bacterial diseases. Apart from public health impacts, residuals losses in the food processing industry ((Botsoglou and Fletouris, 2001).

Deriving adequate withdrawal intervals for extralabel use of veterinary topical products is difficult because of the limited published data and even data for approved drugs are usually proprietary. Therefore, approved products and doses labeled for the specific indication at hand should be used and respected and label withdrawal period should be adhered to (Baynes *et al.* 1997). When determining whether topical application of certain chemicals may violate tolerance levels in meat, milk, and eggs, the veterinarian is often limited to empyreal data. In the decision-making process, factors, such as type of drug formulation used, method of topical application, presence of hair or wool, environmental conditions, and animal species treated, should be considered. In many cases, a conservation estimate for the slaughter withdrawal interval can be derived, despite the data gaps. Such recommendations should not be used for routine extralabel use, but are meant to apply to situations in which the drug has been used and human food safety concerns must be addressed.

In al-Ahsa, Saudi Arabia, a survey was conducted on 1676 chickens collected from retail shops (Natafa) between 1993 and 1994. It was found that 64% of the collected samples contained antibiotics, namely Oxytetracyline (62%), Sulphadimidine (24%) and Ampicillen (9%). Unfortunately, concentrations of antibiotic residues were above the MRLs allowed by the WHO/FAO committees. In the same study, no antibiotics could be detected in mutton (Homida, 1995). In another survey of Saudi markets in Riyadh, Dammam, Jeddah and Al-Ahsa for antibiotic residues between 1998 and 1999, violative concentrations residues were detected in

chicken and mutton when compared to that of the international tolerance limits (Homida, 1999).

Chemical residues of human foodstuffs in sacrificed slaughtered animals are not yet investigated. Between 60-85% of the human daily intakes of chemical residues arise from human foodstuffs of animal origin. Therefore, this study was planned to carry out a survey for certain antibacterial residues in those particular food classes in sacrificed slaughtered camels, sheep and cattle during the season of haj (1423H).

Materials and methods

Sampling

Forty samples from each of semimembranous muscle as well as liver tissues were collected from sacrificed carcasses of camels, cattle and sheep. The samples were stored by freezing until subjected to analysis.

Antibacterial drug residues

Bacterial growth inhibition assay

The earliest methods used for the detection of antimicrobial residues in foods were based on the detection of growth inhibition of various sensitive bacterial strains. This was carried out in this study using *Bacillus subtilis*, gram- positive spore forming bacteria for the assay (Bennett et al., 1966 and Atef et al., 2000). The test culture was a reference strain from the American type culture collection (ATCC 6633). The purity of the bacterial culture was checked by the routine morphological, cultural and biochemical methods. To perform the antibacterial assay, three to five colonies of 24 hour *B. subtilis* culture on brain heart infusion agar (Oxoid Ltd., Hampshire, UK) were picked up and inoculated into 5 ml of Muller-Hinton broth (Oxoid Ltd., Hampshire, UK). The inoculated broth was incubated until its turbidity matched that of McFarland tube No. 0.5. A sterile cotton swab was soaked into the broth and used to completely seed a Mueller-Hinton Agar (Oxoid Ltd., Hampshire, UK). The plates

were left at room temperature for 5 to 10 minutes. In case of tissue samples, a sterile cork porer was used to cut off around pieces (8 mm in diamater). The tissue pieces were gently fixed onto the agar surface of the inoculated plates keeping the pieces apart by about 2 cm from each other and 1.5 cm from the edge of each plate. On the other hand, liquid samples (tissue exudates) were loaded into wells prebunched in the agar (six wells "8 mm in diameter"/ 90 mm plate). Each well was charged with 0.1 ml of the liquid sample. The prepared plates were incubated at 37°C overnight (16-18 hrs). The inhibition zones around the wells or tissue pieces were measured using a transparent ruler. Samples with inhibition zone ≥ 2 mm were considered positive for presence of antibacterial agent(s). Samples with inhibition zone of 1-2 mm were considered as suspects, while those with inhibition zone ≤ 1 mm were considered negative for presence of antibacterial agent(s).

Qualitative and quantitative analysis of the residues

Samples that were found positive for presence of antibacterial drug residues (with the bacterial inhibition assay) were analyzed for type as well as level of the drug(s). Tissues were tested for the most common drugs that are routinely used in the veterinary therapy which are tetracyclines, ampicillin, amoxycillin, erythromycin and sulphonamides. The employed analytical methods differ according to the estimated drug. Samples were prepared as described by many investigators (Abdennebei, 1994, Rose et al., 1997, Sorensen et al., 1997;; and Carson et al., 1998). For multiple Tetracycline residues (tetracycline, oxytetracycline and chlorotetracycline), the chromatographic method of Carson et al. (1998) was carried out in which, metal chelate affinity columns were utilized. Concerning amoxycillin and ampicillin, the method of Rose et al. (1997) was employed. In such a method high pressure liquid chromatography (HPLC) analysis was performed on meat and liver extracts and the readings were recorded using UV of 325 nm wavelength. Data capture and peak height calculation will be performed using Turbochrom V3 software (PE Nelson). Standard solutions of derivatized amoxicillin and ampicillin were prepared in black tissue extract to counter any observed quenching effect for method validation. Determination of erythromycin was carried out according to the methods described by Baggot and Gringerich (1976) and Tsujik and Goetz (1978). SQNa was estimated in the tissues employing the method of Abdennebei (1994).

Results and Discussion

It has been well known that human consumption of animal-originated food containing antibacterial residues might have deleterious effects on human health. This is when the residues exceed the levels that can be tolerated by the consumer body biotransformation systems. There are different limits for different agents and these limits are identified as the MRLs. In a report of the WHO (1999), legislations for antibacterial drug residues in human foods of animal origin were tabulated. The report was agreed up on by the committee of a WHO/FAO joint meeting that was held in Rome in 1998. The meeting evaluated certain veterinary drug residues in food. The legislations were expressed in acceptable daily intake (ADI) and MRLs. Both values differed according to the type of food and the animal species.

This reflects the importance of minimizing the consumption of antibacterial residues that might be present in human food of animal origin. This concept has been considered with more interest especially with the intensive animal breeding and the consequent increased usage of antibacterial drugs for treatment and prophylaxis.

The present study was planned to detect the presence and levels of antibacterial residues in meat and livers of sacrificed slaughtered camels, sheep and cattle. Meat and liver samples were collected from carcasses directly after slaughter. The results of the antibacterial drug residue tests in the examined samples are depicted in table (1). Out of 40 muscles samples from sacrificed carcasses of camel, only one sample (2.5%) was positive for the presence of antibacterial drug residues. While, out of 40 liver samples from the same carcasses, two samples (5%) were positive for the presence of antibacterial drug residues. This was based on the results of the bacteriological assays. The antibacterial substance(s) in one meat samples could not be identified. On the other hand, SQNa and CAP were detected in the two liver samples of sacrificed carcasses of camels with concentrations of 0.04 and 0.03 mg/kg, respectively. The limits of SQNa and CAP residues found in samples of liver were significantly lower than the MRLs obtained by the Codex Alimentarius (WHO,1998 &1999). These results suggest that, on the whole, sacrificed carcasses of camels probably possess no serious health hazard to the consumers. However, it should be noted that the finding of CAP residue (albeit in small concentration) should be taken seriously, as this antibacterial drug is hardly employed in veterinary practice, and its

use in human medicine should be restricted to a few life-threatening conditions such as meningitis. Nowadays, CAP is not recommended for animal therapy and has been replaced by a less toxic antibiotic, florfenicol.

In sacrificed sheep carcasses, antibacterial residues could not be detected in muscle tissues. While, out of 40 liver samples only one sample (2.5%) was contained SQNa and unknown inhibitory substance. The concentration of SQNa in liver tissue was (0.02 mg/kg) below the MRLs.

Analysis of muscles and liver samples (40 for each) collected from sacrificed cattle carcasses for presence of antibacterial residues, the results revealed that no antibacterial residues were detected in any of the examined samples. This indicates the safety of the cattle meat and/or liver, as far as antibacterial drugs are concerned.

The incidence as well as the levels of antibacterial residues recorded during the period of this study are comparatively much below the permissible MRLs. Moreover, the present results were lower than the previous studies (Homida, 1995 and 1999) which carried out in another localities in Saudi Arabia, this may be attributed to the differences in the sources of the samples examined in the other studies.

The present study reflects the awareness of the authorities, veterinarians and the community about the problem and its impact on the public health. Conclusively, if antibacterial drug residues are concerned and according to our study data, it can be postulated that carcasses of sacrifice slaughtered animals are safe for human consumption.

Table (1): The incidence and concentrations of antibacterial residues in sacrificed carcasses of animals slaughtered during season of Haj (1423H).

Species	Muscle				Liver					MRLs*** (mg/kg)	
	No. of samples	No. of +ve samples	%	Type*	No. of samples	No. of +ve samples	%	Type*	concentration (mg/kg)	Muscle	Liver
Camels	40	1	2.5	μ**	40	2	5	SQNa CAP	0.04 0.03	0.1 0.2	0.1 0.6
Sheep	40	ND	-	-	40	1	2.5	SQNa μ**	0.02	0.1	0.1
Cattle	40	ND	-	ND	40	ND	-	ND	ND	-	-

* Tetracycline, Oxytetracycline, penicillin, amoxicillin and ampicillin could not be detected in the examined samples
** Unknown inhibitory substance
SQNa = Sulphaquinoxalizin sodium
CAP = Chloramphenicol
ND = not detected
***MRLs = Maximum Residue Limits according to the Codex Alimentarius (WHO,1998 &1999).

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